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SUFFIELD REPORT

NO. 427

TRACE ANALYSIS OF MYCOTOXINS IN BLOOD BY CAPILLARY COLUMN-AMMONIA CHEMICAL IONIZATION MASS SPECTROMETRY (U)

by

P.A. D'Agostino, L.R. Provost and D.R. Drover

PCN 13E20

April 1986

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD, RALSTON, ALBERTA

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ABSTRACT

Capillary column-ammonia chemical ionization mass spectrometry was found to be an excellent tecnhique for the trace detection and identification of underivatized trichothecene mycotoxins in blood. Abundant $(M+H)^+$ and/or $(M+NH_4)^+$ pseudo-molecular ions were observed for T-2 toxin, HT-2 toxin, T-2 triol, diacetoxyscirpenol, deoxynivalenol and verrucarol under the conditions suggested. Detection limits in the 0.7 to 5.1 ng/g range were possible due to the specificity of this method. This method was successfully applied to the analysis of blood samples spiked with an undisclosed number of mycotoxins during a recent interlaboratory exercise. T-2 toxin, HT-2 toxin, diacetoxyscirpenol and verrucarol were detected in these samples at levels ranging from 2 to 180 ng/g.

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INTRODUCTION

Trichothecene mycotoxins are a group of naturally occurring sesquiterpenoid compounds produced by various species of imperfect fungi. Figure 1 illustrates the common ring structure and functional groups associated with a number of mycotoxins.

The defence science community recently became interested in mycotoxins due to reports of their alleged use as chemical warfare agents in Southeast Asia (1 – 3). These 'yellow rain' allegations have been supported by the findings of Rosen and Rosen (4) and Mirocha (5). Rosen and Rosen (4) found the mycotoxins deoxynivalenol (DON), diacetoxysirpenol (DAS), T-2 toxin (T2) and zearalenone (F2) in a sample of yellow powder scraped from vegetation by Hmong resistance fighters in the Phou Bia area. Mirocha (5) detected DAS, DON and F2 in leaves, water and yellow powder obtained from 'yellow rain' attack sites in Southeast Asia. In addition T2 and HT-2 toxin (HT2), a metabolite of T2 in animals, were found in biological fluids of alleged victims (5).

The need for routine agricultural monitoring and more recently the threat of mycotoxin use in chemical warfare applications has resulted in the development of a number of analytical techniques for their detection. These methods have been reviewed by Black (6) and include thin layer chromatography, high performance liquid chromatography (HPLC) (7 – 12), supercritical fluid chromatography (SFC) (13 – 15), tandem mass spectrometry (16, 17) and gas chromatography (GC) (4, 5, 18 – 36). Gas chromatography-mass spectrometry (GC-MS) methods are often favoured due to instrument availability and the sensitivity and specificity of these methods. Most GC-MS methods utilize derivatization with trimethylsilyl acetamide, heptafluorobutrylimidazole or another derivatizing agent prior to analysis. However, a number of laboratories (17, 34, 35), including our own (37), recently demonstrated the ability to detect mycotoxins in an underivatized state by using fused silica capillary columns.

We report the evaluation of several clean-up procedures (32, 38, 39) and the development of a capillary column GC-MS method for the trace determination of mycotoxins in blood that does not require mycotoxin derivatization. Mass spectral determinations were performed under ammonia chemical ionization conditions, a technique recently reported for HPLC-MS (8, 10) and SFC-MS (14) analysis of mycotoxin standards. Abundant $(M+H)^+$ and/or $(M+NH_4)^+$ pseudo-molecular ions were observed under the conditions selected with considerably less fragmentation than was observed during electron-impact ionization. This method was applied to the analysis of twenty blood samples spiked with mycotoxins in the 0 to 500 ng/g range as part of a TTCP exercise designed to evaluate laboratory procedures. The blood extracts were screened for the presence of T2, HT2, T-2 triol, DAS, DON and verrucarol (VER) by

capillary column-ammonia chemical ionization mass spectrometry. A number of mycotoxins including T2, HT2, DAS and VER were detected at levels ranging from 2 to 180 ng/g. The specificity of this method resulted in excellent detection limits (0.7 to 5.1 ng/g) for these mycotoxins in blood. The results of the other laboratories are presented for comparative purposes.

EXPERIMENTAL

a. Blood Samples

Twenty 5 mL blood samples spiked with 0 to 500 ng/g of an undisclosed number of mycotoxins and 100 mL of blank blood were supplied by the Centre for Disease Control, Atlanta, GA. The blood samples were stabilized with heparin and 0.5% sodium fluoride prior to delivery and stored at 4°C for four months prior to clean-up and analysis.

b. Blood Clean-Up

Three schemes for blood clean-up (32, 38, 39) were selected for preliminary study. After assessment, a slightly modified UK scheme was selected (32). A brief description of the clean-up scheme is presented in Figure 2.

HPLC grade solvents and Milli-pore filtered distilled water were used for sample clean-up. The sodium sulphate used to dry the methylene chloride extracts was BDH analytical grade. C_{18} Sep-Pak cartridges were purchased from Waters Associates (Milford, MA). The centrifuge tubes were borosilicate glass with PTFE-lined tops. An all glass-PTFE Buchi mini-rotovap was used to concentrate the samples.

c. Mycotoxin Standards

The mycotoxin standards were prepared in "HPLC grade" acetone and stored in PTFE-lined screw-topped glass vials. DAS, DON and T2 were purchased from Myco-Labs, Chesterfield, MO. VER, HT2 and T-2 triol were obtained from Sigma Chemicals, St. Louis, MO.

d. Instrumental Analysis

Gas chromatographic analyses were carried out on two different instruments. Most GC-FID chromatograms were obtained using a Varian 6000 (Varian Associates, Georgetown, Ont.) gas chromatograph equipped with a J+W on-column injector (J+W Scientific, Rancho Cordova, CA). Varian 6000 GC conditions follow: 15 m × 0.32 mm I.D. 0.25 μm J+W DB-1 capillary column; 35 cm/s He; ambient temperature injection followed by a temperature program 75°C (2 min) 10°C/min to 300°C (10 min). Additional GC-FID chromatograms were obtained using a Varian 3700 equipped with an on-column injector of our own design (40). Varian 3700 conditions follow: 15 m × 0.32 mm I.D. 0.25 μm J+W DB-5 capillary column; 35 cm/s He; temperature program 50°C (2 min) 10°C/min to 300°C (8 min).

Capillary column GC-MS analyses were performed with a VG Micromass 70/70E mass spectrometer (VG Analytical, Wythenshawe, UK) equipped with a Varian 3700 gas chromatograph. Chromatographic conditions were the same as used for Varian 3700 GC-FID study.

The electron-impact and chemical ionization operating conditions used during all GC-MS analyses are listed in Table I. The ions monitored for each mycotoxin during accelerating voltage selected-ion-monitoring (SIM) analysis are listed in Table II.

RESULTS AND DISCUSSION

a. Gas Chromatography

Most of the gas chromatographic analysis methods reported use sample derivatization prior to mycotoxin detection. Heptafluorobutyryl derivatives have been used with electron capture detection (ECD) to quantitate mycotoxins in grains (19, 22, 24, 28) and plasma (27, 33). Trimethysilyl derivatization has been reported for mycotoxin quantitation in food and/or feeds (21, 30, 31) with flame ionization detection (FID). The elimination of derivatization was attractive since it would save time during sample workup and minimize the potential losses inherent in an additional handling step. In addition concerns about derivative stability have also been expressed and several reports (19, 22, 24, 28) suggest same day analysis. For these reasons a GC method was developed to analyse mycotoxins in an underivatized state.

During preliminary capillary column GC-FID analysis, the mycotoxin standards displayed poor or inconsistent peak shape. The high polarity of the methanol solvent was suspected since the standards were believed to be ⩾ 95% pure. In an attempt to provide better chromatographic peak shape, methanol solutions of the standards were co-injected with a chloroform plug (41). The presence of chloroform resulted in better peak shape, particularly in the case of verrucarol. Further solvent investigation indicated that excellent peak shape and increased area counts per nanogram could be obtained with acetone. Figure 3 illustrates typical capillary column GC-FID chromatograms obtained with methanol, methanol with a chloroform plug and acetone as the solvent. Acetone was selected as the solvent for subsequent investigations on the basis of this chromatographic evidence.

Figure 4 illustrates a capillary column GC-FID chromatogram obtained for the six mycotoxins used in this study. T2, HT2 and T-2 triol co-eluted on the DB-1 column. However, slight differences in retention time on the DB-5 column and more importantly marked mass spectral differences, permitted independent detection of these three mycotoxins by capillary column-ammonia chemical ionization mass spectrometry. The DB-5 retention order was the same as reported by Miles (35); T2, then HT2 and finally T-2 triol. Longer capillary columns should be employed for the resolution of these three mycotoxins with less specific detectors. More polar columns are not recommended since these compounds already elute at a high temperature.

A standard containing VER, DAS and T2 was prepared to evaluate FID detector linearity. Correlation coefficients of 0.993, 0.989 and 0.990 were observed for VER, DAS and T2 respectively over the 10 to 100 ng range. The detection limit for T2 was 200 pg (S/N ⩾ 3:1) based on 500 pg injections. Similar detection limits were expected for the other mycotoxins since their FID response curves were similar. This level of detection was not sufficient for ng/g detection in blood but could be considered for μg/g levels in blood or lower levels in less complex matrices.

b. Blood Clean-Up Scheme Evaluation

Three clean-up schemes were evaluated for the concentration of mycotoxins from blood. The clean-up schemes were developed by Agriculture Canada (39), and chemical defence researchers in the United States (38) and the United Kingdom (32). Each

scheme was evaluated with 5 mL aliquots of blank blood stabilized with heparin and sodium fluoride. The capillary column GC-FID chromatograms, illustrated in Figure 5, exhibited roughly the same impurity content. Contamination was minimal when 5 mL of water was taken through the clean-up schemes.

The Agriculture Canada scheme was not selected since it was designed exclusively for T2 analysis. We selected the UK scheme over the US scheme since the US scheme was undergoing revision and required more sample handling.

The UK scheme (32) was initially evaluated for recovery efficiency with 5 mL of water spiked with 10, 2.5 and 1 μg of VER, DAS and T2. These experiments were necessary since efficiency data were not available prior to evaluation. Recoveries from water, listed in Table III, were in the 42 – 63%, 52 – 71% and 61 – 76% range for VER (and a derivative of VER), DAS and T2 respectively.

A peak with a slightly longer retention time was noted in place of or with VER in the spiked water extractions. The electron-impact mass spectrum of this compound was somewhat ambiguous since there was no evidence of molecular ion presence. However both $(M+H)^+$ and $(M+NH_4)^+$ pseudo-molecular ions were observed during capillary column-ammonia chemical ionization mass spectral analysis. It was evident from the isotopic pattern that a chlorine was present and that the molecular weight of the unknown was greater than verrucarol by 34 mass units. It appears that one hydrogen was replaced by chlorine. This compound was tentatively identified as a chlorinated-verrucarol on the basis of this and fragmentation evidence. Figure 6 illustrates the mass spectra obtained and Figure 7 illustrates a typical capillary column GC-FID chromatogram of a spiked water extract containing this compound. It was determined, through spiking experiments, that chlorinated-verrucarol was formed in small amounts during the methylene chloride extraction clean-up step. The subsequent sodium sulfate drying step appeared to increase conversion to chlorinated-verrucarol. Total disappearance of verrucarol after clean-up of spiked water was not uncommon.

C_{18} Sep-Pak recoveries were investigated with the same VER, DAS and T2 mycotoxin standard. An additional 1 mL of 90% methanol/10% water was added to the UK scheme to improve recovery at this step. Table IV presents the percentage recovery data for each mycotoxin after C_{18} Sep-Pak elution.

T2 was found to disappear on standing in water for 48 hours. Comparison of T2 peak area with the same amount (10 μg) left in methanol indicated a recovery of 78 ± 5%. The presumed hydrolysis of T2 could be significant for T2 left standing after the C_{18} Sep-Pak clean-up step.

A final evaluation of the UK clean-up scheme was done by spiking blank blood with DAS, VER and T2. DAS and T2, spiked at the 10 μg level, were recovered at 30 ± 10% (n = 3) and 50 ± 10% (n = 3) respectively. Figure 8 illustrates the ability to quantitate DAS and T2 in blood extracts by capillary column GC-FID at μg/g levels. At these levels both DAS and T2 can also be confirmed during full scanning GC-MS operation in the electron-impact ionization mode. Figure 9 illustrates a total-ion-current chromatogram and the resulting electron-impact mass spectra for both DAS and T2. The electron-impact mass spectra of the principle impurities (A to D) are illustrated in Figure 10. Identifications have not been made at this time. Similar recoveries of T2 and DAS were observed during subsequent capillary column-ammonia chemical ionization mass spectral analysis of the 2.5 and 1 μg spiked blood samples. Recoveries from the UK clean-up scheme were similar to those recently published (32).

Neither verrucarol or chlorinated-verrucarol were observed in the spiked blood by capillary column GC-FID or GC-MS (EI or CI) when the UK clean-up scheme was employed. Decomposition or reaction during blood clean-up was suspected since it was possible to detect VER and chlorinated-verrucarol during co-injection with a blood blank extract. Subsequent discussions with R. Black, author of the UK scheme (32), indicated that the methylene chloride extraction step was included only to prevent possible T2 hydrolysis. It was therefore decided that this step would be omitted for seven (randomly selected) of the provided blood samples in an attempt to demonstrate the detection of verrucarol in blood.

c. Gas Chromatography-Mass Spectrometry

Capillary column GC-MS investigations were initially done under electron-impact ionization conditions. The mass spectra obtained for the six mycotoxins, illustrated in Appendices 1 and 2, all exhibited considerable fragmentation and little or no molecular ion information. The electron-impact mass spectra were similar to published results (42).

Capillary column GC-MS (EI) was useful at μg/g mycotoxin levels in blood under full scanning conditions (Figure 8). However, for trace detection there were considerable problems due to the lack of molecular ion and higher molecular weight fragmentation ions. Selected-ion-monitoring of lower molecular weight fragmentation ions was not considered sufficient for confirmation purposes due to the potential for interference.

Reports of abundant $(M+H)^+$ and/or $(M+NH_4)^+$ pseudo-molecular ions during SFC-MS and HPLC-MS (8, 10, 14) led to the development of a capillary column-ammonia chemical ionization mass spectrometry method. Recent ammonia CI experiences (43 – 46) suggested the use of maximum CI gas pressures and lower source temperatures. Considerable peak tailing, probably due to the low volatility of the mycotoxins, was observed at source temperatures below 140°C. Higher source temperatures reduced the peak tailing. However, the relative abundance of the pseudo-molecular ions dropped off substantially. Table V illustrates these findings for T2, DAS and VER at source temperatures ranging from 140 to 220°C. A setting of 160°C was selected as it provided reasonable peak shape and good pseudo-molecular ion abundance.

Figures 11 and 12 illustrate the ammonia chemical ionization mass spectra of the six mycotoxins studied. Abundant $(M+H)^+$ and/or $(M+NH_4)^+$ pseudo-molecular ions were observed for all the mycotoxins with considerably less fragmentation than under EI conditions (refer to Appendices 1 and 2).

Full scanning CI mass spectra could be obtained with as little as 10 ng of mycotoxin. Selected-ion-monitoring afforded standard detection limits of 20, 30, 80, 130, 530 and 600 pg ($S/N \geq 3:1$) for T2, DAS, HT2, VER, T-2 triol and DON respectively, based on the detection of two characteristic ions. These detection limits would improve by a factor of four or five if tentative identification, based on only one ion, was considered. The detection limits were in the same ranges as those found by Miles (35) and Lau (17) during underivatized GC-MS analysis with Townsend discharge CI sources. Miles reported detection limits in the 50 to 375 pg range, based on the detection of two ions, and Lau was able to detect mycotoxins in the 10 to 305 pg range during parent/daughter MS/MS operation.

d. Application

Twenty blood samples, spiked with mycotoxins in the 0 to 500 ng/g range were taken through the UK clean-up scheme (Figure 2) along with blood blanks. Seven of the samples (randomly selected) were not taken through the methylene chloride/sodium sulfate step. All clean-up extracts, including blanks, were screened for the presence of T2, HT2, T-2 triol, DAS, VER and DON by the capillary column-ammonia chemical ionization mass spectrometry method developed. Confirmation was based on both a GC retention time match and relative ion abundance match of the SIM traces with mycotoxin standards. External standard quantitation was used for the duplicate analysis of each sample extract.

T2, HT-2, DAS and VER were detected at levels ranging from 2 to 180 ng/g in the blood clean-up extracts. No false positives were noted in the blood blanks. Figures 13, 14, 15 and 16 illustrate the trace detection of T2, HT-2, DAS and VER respectively in several clean-up extracts.

Detection limits were slightly better in samples that did not include the methylene chloride/sodium sulfate step due to decreased background currents. We estimate sample detection limits ($S/N \geq 3:1$) of 0.7 and 2.0 ng/g, and 3.6 and 5.1 ng/g with and without this step for T2 and DAS respectively. VER and HT2 were detectable at 3.3 ng/g and 4.6 ng/g respectively in samples not taken through the methylene chloride/sodium sulfate step.

These detection limits were lower than those reported for the determination of T2, HT2 and DAS in porcine plasma by thermospray HPLC-MS (8) and for the determination of T2 in plasma by GC-ECD (27, 33). Similar detection limits were reported for the determination of HT2 in plasma by GC-ECD (33). The UK capillary column GC-MS method for mycotoxins in blood (32) was slightly more sensitive and capable of detection in the 0.1 to 10 ng/g range. The excellent detection limits, attributed to the ionization selectivity of ammonia chemical ionization, resulted in sample detection limits an order of magnitude lower than many of the methods for mycotoxins listed in Table VI.

Table VII lists the mycotoxins found during analysis of the blood samples provided. The DRES results for DAS, T2 and VER are compared with laboratories A and B in Table VIII. The nivalenol results from laboratory B are not shown since it was the only laboratory to screen for this compound. Given the semiquantitative emphasis of the exercise, the results of all laboratories were considered good.

We could not reliably detect VER in blood extracts after clean-up with the UK scheme. It was detected in only two samples taken through the clean-up without the methylene chloride/sodium sulfate step. It appears that omission of this step does not always allow VER detection since none was found in the more concentrated 'C' series samples. However, omission of this step did appear to lead to partial hydrolysis of T2 in the sample spiked at 200 ng/g. HT2, a hydrolysis product of T2, was detected at a trace level in sample A3.

It was noted during blood extract analysis that the peak shape of standards deteriorated (i.e., tailing increased) with the number of extracts analysed. This may be remedied by cutting small portions of column at the injector end or by using short disposable lengths of column prior to the analytical GC column. However, peak shape did not deteriorate during analysis of mycotoxins in the blood extracts. This was evident by the excellent shape observed during SIM analysis (Figures 13, 14, 15 and 16). Presumeably, the presence of other materials in the blood extracts mask the active column sites. Standards may be prepared in the blank blood to take advantage of this effect.

CONCLUSIONS

The capillary column-ammonia chemical ionization mass spectrometry method developed was found to be an excellent technique for the trace detection of underivatized trichothecene mycotoxins in blood. Abundant $(M+H)^+$ and/or $(M+NH_4)^+$ ions were observed for T-2 toxin, HT-2 toxin, T-2 triol, diacetoxyscirpenol, deoxynivalenol and verrucarol under the conditions suggested.

This method was used successfully for the analysis of mycotoxins in blood during a recent international training exercise. The excellent detection limits (0.7 to 5.1 ng/g) of the method allowed the determination of mycotoxins in the 2 to 180 ng/g range.

The UK clean-up scheme used for the concentration of mycotoxins from blood was evaluated and recovery efficiencies were similar to those obtained during its development. Less GC-MS background was observed when the methylene chloride/sodium sulfate step was not included in blood clean-up. However, omission of this step appears to enhance T-2 toxin hydrolysis.

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Table I

CAPILLARY COLUMN GC-MS OPERATING CONDITIONS

OPERATING PARAMETER	ELECTRON IMPACT	AMMONIA[1] CHEMICAL IONIZATION
Accelerating Voltage	6 kV	6 kV
Electron Energy	70 eV	50 eV
Emission	100 μA	500 μA
Source Temperature	185 – 190°C	160°C
Source Pressure	2×10^{-6} torr	5×10^{-5} torr (near source)
Resolution (10% valley)	1000	1000
GC-MS Interface	direct @ 230°C	direct @ 230°C
Scan Function and Rate	550 to 20 u, expontial down, 1 s/decade	550 to 20 u, expontial down, 1 s/decade See Table II for SIM Conditions

1 Anhydrous grade (99.99%) (Liquid Carbonic, Scarborough, Ont.)

Table II

IONS MONITORED DURING SELECTED-ION-MONITORING[1] GC-MS (CI)

MYCOTOXIN	ION MONITORED $m/_z$	ION IDENTITY	DWELL TIME (ms)
T2	480.97[2]		100
	484.25	$(M+NH_4)^+$	300
	485.25	$(M+1+NH_4)^+$	300
HT2	430.97[2]		100
	442.24	$(M+NH_4)^+$	300
	443.24	$(M+1+NH_4)^+$	300
T-2 triol	380.98[2]		100
	383.21	$(M+H)^+$	300
	384.21	$(M+1+H)^+$	300
VER	230.99[2]		100
	249.14	$(M-OH)^+$	300
	267.14	$(M+H)^+$	300
DAS	380.98[2]		100
	384.20	$(M+NH_4)^+$	300
	385.20	$(M+1+NH_4)^+$	300
DON	292.98[2]		100
	297.14	$(M+H)^+$	300
	314.16	$(M+NH_4)^+$	300

1 Resolution (10% valley) of 300 – 500

2 PFK reference lock mass

Table III

MYCOTOXIN RECOVERY FROM WATER USING UK CLEAN-UP SCHEME

MYCOTOXIN SPIKE μg (μg/g BLOOD)	% RECOVERY[1]		
	VER[2]	DAS	T2
10 μg (2 μg/g)	50 ± 4	50 ± 4	64 ± 6
10 μg (2 μg/g)	61 ± 4	67 ± 6	76 ± 4
2.5 μg (0.5 μg/g)	63 ± 2	64 ± 2	76 ± 4
2.5 μg (0.5 μg/g)	52 ± 2	55 ± 3	61 ± 3
1.0 μg (0.2 μg/g)	47 ± 7	72 ± 11	61 ± 11
1.0 μg (0.2 μg/g)	42 ± 6	52 ± 3	72 ± 7
RANGE:	**42 – 63%**	**52 – 72%**	**61 – 76%**

1 Mean ± SD (n = 3) based on GC-FID data

2 Chlorinated verrucarol and verrucarol recovery

Table IV

RECOVERY OF T2, DAS AND VER FROM C_{18} SEP-PAK STEPS USED IN UK CLEAN-UP SCHEME

C_{18} SEP-PAK STEP	% RECOVERY		
	VER	DAS	T2
2 mL H_2O wash	0	0	0
2 mL 40% MeOH 60% H_2O	25	0	0
2 mL 90% MeOH 10% H_2O	63	81	75
*1 mL 90% MeOH 10% H_2O	13	19	25
*1 mL 90% MeOH 10% H_2O	0	0	0

* Steps were not included in the UK clean-up scheme (32)

Table V

DEPENDENCE OF RELATIVE ION INTENSITY ON SOURCE TEMPERATURE FOR T2, DAS AND VER DURING CAPILLARY COLUMN - AMMONIA CHEMICAL IONIZATION MASS SPECTROMETRY

COMPOUND	m/z	% RELATIVE INTENSITY				
		140° C	160° C	180° C	200° C	220° C
T2	484 $(M+NH_4)^+$	100	100	58	35	20
	364	12	16	29	23	18
	122	50	95	100	100	100
DAS	384 $(M+NH_4)^+$	100	100	100	72	42
	306	13	24	43	47	62
	109	40	35	74	100	100
VER	284 $(M+NH_4)^+$	30	16	6	4	ND
	267 $(M+H)^+$	78	35	27	17	10
	249	100	100	100	100	100
	231	52	60	58	54	53

ND – Not Detected

Table VI

MYCOTOXIN DETECTION LIMITS

METHOD	DERIVATIVE USED	MYCOTOXIN(S)	DETECTION LIMIT (ng/g)	SAMPLE TYPE	REFERENCE
HPLC - refractive index	none	T2, HT2	1000	cereals	7
Thermospray HPLC - MS	none	T2, HT2, DAS, DON, F2, T-2 tetraol	1 - 10 ng (not given in ng/g)	porcine plasma and urine	8
HPLC - MS (NICI)	none	NIV, DON	5 – 10	cereals	11
MS / MS	none	DON, F2	100 – 1000	grains	16
GC - ECD	heptafluorobutyryl	T2, DAS	25 (DAS) 100 (T2)	corn, feed	19
		DON	10	grains	22
		DON	50	grains	24
		T2, HT2, DAS	100 (DAS, T2) 50 (HT2)	cereals	28
		T2	25	plasma	27
		T2, HT2	30 (T2) 5 (HT2)	plasma	33

.../2

Table VI

MYCOTOXIN DETECTION LIMITS (Continued)

METHOD	DERIVATIVE USED	MYCOTOXIN(S)	DETECTION LIMIT (ng/g)	SAMPLE TYPE	REFERENCE
GC-FID	trimethylsilyl	5 toxins including T2, HT2, DON	50 – 100	feeds	21
		10 toxins including T2, HT2, DAS, DON	50	food, feeds	30
		5 toxins including T2, HT2, DAS	100 – 1000	food	31
GC-MS (EI)	trimethylsilyl	T2	5	maize	23
		T2, HT2, DAS, F2	20 – 50	corn	29
GC-MS (CI)	trimethylsilyl (also for EI study)	T2	3	milk	20
	trifluoroacetate (also for EI study)	HT2, T2, DAS, DON, F2	1	yellow rain	5
	pentafluoropropionyl	11 toxins including T2, HT2, DAS, DON	0.1 – 10	blood	32
	none	DON	130 (full scan)	grains, food	34
	none	T2, HT2, DAS, VER	0.7 – 5.1	THIS STUDY	

UNCLASSIFIED
UNCLASSIFIED

Table VII

RESULTS OF BLOOD ANALYSIS AT DRES

SAMPLE NUMBER	MYCOTOXIN CONCENTRATION (ng/g)							
	T2	AVG	DAS	AVG	VER	AVG	HT2	AVG
	(measurements)		(measurements)		(measurements)		(measurements)	
A1	(135, 115, 124)	125	(11, 12)	12		ND[6]		ND[7]
A2	(112, 120)	116	(12, 13)	13		ND[6]		ND[7]
A3*	(142, 118)	130	(14, 9.9)	12		ND[6]	(4.3, 4.5)	4.4
A4	(179, 163)	171	(10, 9.7)	9.9		ND[6]		ND[7]
B1	(44, 38)	41		ND[3]		ND[6]		ND[7]
B2	(32, 33)	33		2.2[5]		ND[6]		ND[7]
B3	(40, 38)	39		3.1[5]		ND[6]		ND[7]
B4	(30, 31)	31		2.1[5]		ND[6]		ND[7]
C1*	(2.9, 2.7)	2.8	(33, 43)	38		ND[6]		ND[7]
C2	(9.9, 9.9)	9.9	(108, 89)	99		ND[6]		ND[7]
C3*	(9.9, 11)	10.5	(61, 73)	67		ND[6]		ND[7]
C4	(9.3, 9.0)	9.2	(161, 169)	165		ND[6]		ND[7]
D1*		ND[2]		ND[4]		ND[6]		ND[7]
D2		ND[1]		ND[3]		ND[6]		ND[7]
D3		ND[1]		ND[3]		ND[6]		ND[7]
D4*		ND[2]		ND[4]		ND[6]		ND[7]
E1	(2.1, 2.0)	2.1	(34, 42)	38		ND[6]		ND[7]
E2	(2.8, 3.0)	2.9	(46, 55)	50		ND[6]		ND[7]
E3*	(2.5, 2.2)	2.4	(43, 42)	43	(4.1, 5.1)	4.6		ND[7]
E4*	(2.4, 2.3)	2.4	(35, 40)	38	(5.1, 5.4)	5.3		ND[7]

* Methylene chloride extraction step removed from clean-up procedure for this sample

1 $\leq$ 2 ng/g T2 (based on 2 ions; S/N $\geq$ 3:1)

2 $\leq$ 0.7 ng/g T2 (based on 2 ions; S/N $\geq$ 3:1)

3 $\leq$ 5.1 ng/g DAS (based on 2 ions; S/N $\geq$ 3:1)

4 $\leq$ 3.6 ng/g DAS (based on 2 ions; S/N $\geq$ 3:1)

5 Based on the detection of the $(M+NH_4)^+$ ion only

6 $\leq$ 4.6 ng/g VER (based on 2 ions; S/N $\geq$ 3:1)

7 $\leq$ 3.3 ng/g HT2 (based on 2 ions; S/N $\geq$ 3:1)

Table VIII

COMPARISON OF DATA OBTAINED BY DRES* AND LABORATORIES A AND B

SAMPLE NUMBER	MYCOTOXIN CONCENTRATION (ng/g)											
	T2				DAS				VER			
	SPIKE LEVEL	A	B	DRES	SPIKE LEVEL	A	B	DRES	SPIKE LEVEL	A	B	DRES
A 1	200	205	400	125	10	4	12	12	10	15	6	ND
A 2	200	227	470	116	10	5	12	13	10	15	10	ND
A 3	200	224	380	130	10	4	9	12	10	15	6	ND
A 4	200	217	270	171	10	4	11	9.9	10	15	9	ND
B 1	40	24	32	41	2	1	1	ND	2	7.5	1	ND
B 2	40	23	33	33	2	1	1	2.2	2	7.4	1	ND
B 3	40	40	70	38	2	8	1	3.1	2	7.2	1	ND
B 4	40	20	33	31	2	7	1	2.1	2	7.4	1	ND
C 1	10	8.9	8	2.8	200	91	220	38	150	160	160	ND
C 2	10	12	7	9.9	200	138	230	99	150	190	180	ND
C 3	10	6.6	11	10.5	200	94	250	67	150	175	250	ND
C 4	10	12	11	9.2	200	130	230	165	150	184	200	ND
D 1	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
D 2	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
D 3	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
D 4	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
E 1	2	2.9	3	2.1	40	25	40	38	30	31	35	ND
E 2	2	1.9	2	2.9	40	1.8	40	50	30	28	1	ND
E 3	2	2.0	1	2.4	40	21	40	43	30	31	40	4.6
E 4	2	2.7	4	2.4	40	21	40	38	30	25	7	5.3

* Clean-up scheme recovery efficiency not taken into account

ND Not Detected

Class A Trichothecenes

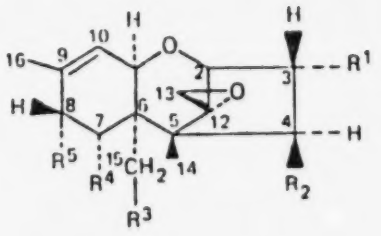
(soluble in non-polar solvents)

Trichothecene (abbr.)	Mol. Wt.	R^1	R^2	R^3	R^4	R^5
Monoacetoxyscirpenol (MAS)	324	OH	OH	OAc	H	H
Diacetoxyscirpenol (DAS)	366	OH	OAc	OAc	H	H
Neosolaniol (NEOS)	382	OH	OAc	OAc	H	OH
T-2 Toxin (T2)	466	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 Toxin (HT2)	424	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
T-2 Triol (T2-T)	382	OH	OH	OH	H	$OCOCH_2CH(CH_3)_2$
Verrucarol (VER)	266	H	OH	OH	H	H
T-2 Tetraol	298	OH	OH	OH	H	OH

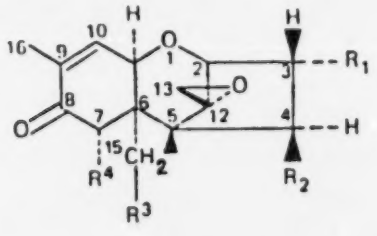
Class B Trichothecenes

(soluble in polar solvents)

	Mol. Wt.	R^1	R^2	R^3	R^4
Deoxynivalenol (DON)	296	OH	H	OH	OH
Deoxynivalenol 15-Acetate (15-ADON)	338	OH	H	OAc	OH
Fusarenon-X (FUS-X)	354	OH	OAc	OH	OH



Basic Δ^9-trichothecene (Class A)



8-Ketotrichothecene (Class B)

Figure 1

MYCOTOXIN STRUCTURES

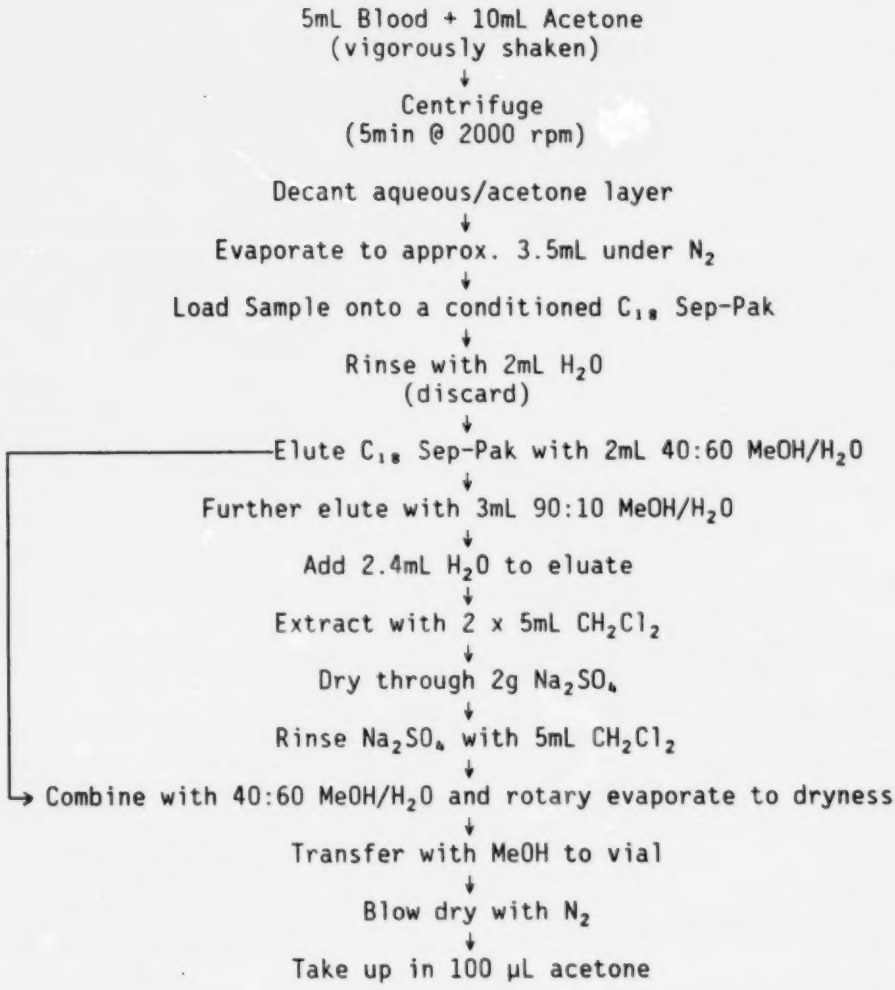

5mL Blood + 10mL Acetone
(vigorously shaken)
Centrifuge
(5min @ 2000 rpm)
Decant aqueous/acetone layer
Evaporate to approx. 3.5mL under N_2
Load Sample onto a conditioned C_{18} Sep-Pak
Rinse with 2mL H_2O
(discard)
Elute C_{18} Sep-Pak with 2mL 40:60 MeOH/H_2O
Further elute with 3mL 90:10 MeOH/H_2O
Add 2.4mL H_2O to eluate
Extract with 2 x 5mL CH_2Cl_2
Dry through 2g Na_2SO_4
Rinse Na_2SO_4 with 5mL CH_2Cl_2
Combine with 40:60 MeOH/H_2O and rotary evaporate to dryness
Transfer with MeOH to vial
Blow dry with N_2
Take up in 100 µL acetone

Figure 2

UK BLOOD CLEAN-UP SCHEME FOR MYCOTOXINS (32)

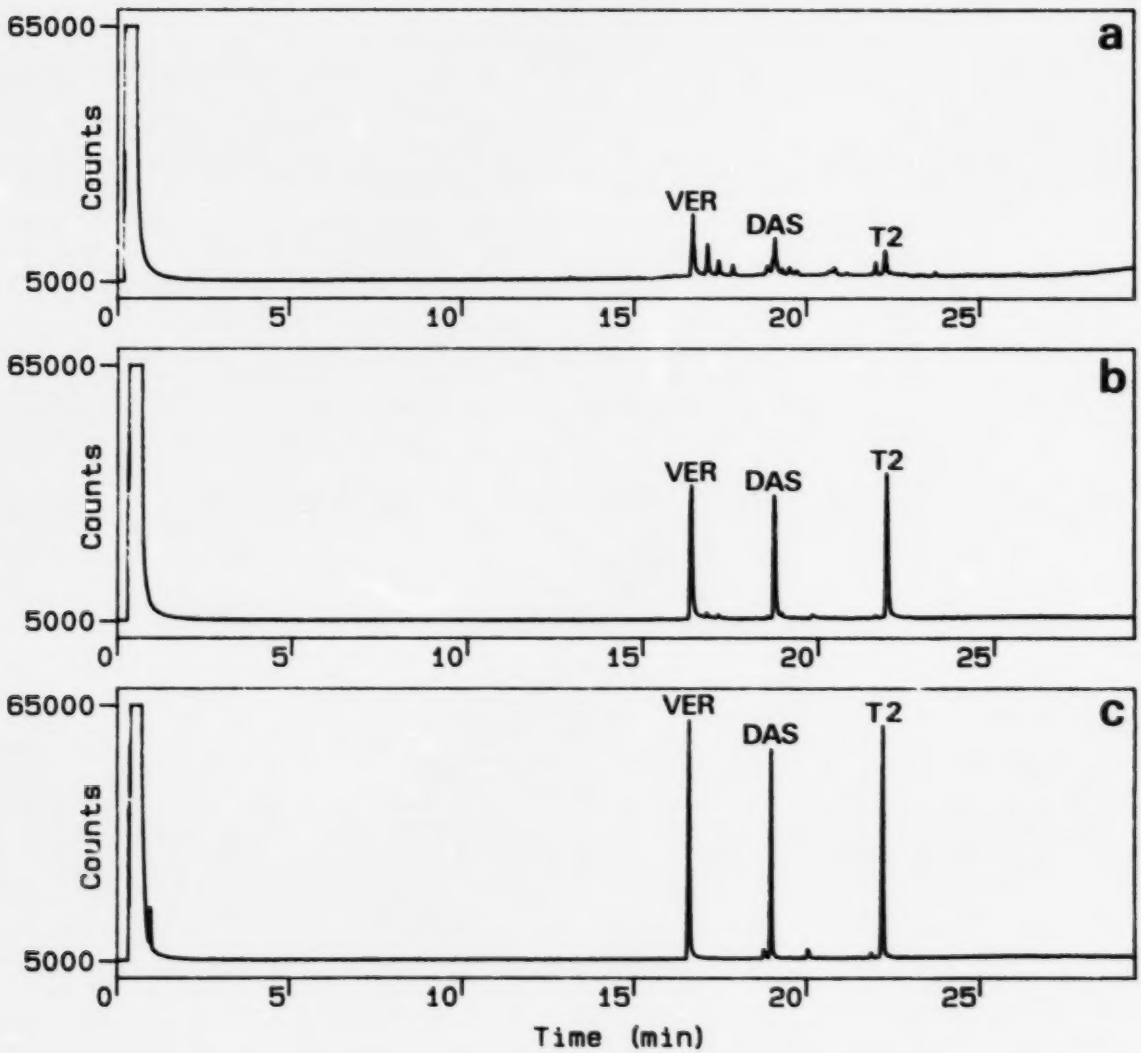


Figure 3

Capillary Column GC-FID chromatograms of 80 ng of VER, DAS and T2 in: a. methanol; b. methanol with a chloroform plug; and, c. acetone (6×10^{-11} amps full scale; 75°C (2 min) 10°/min 300°C (10 min); 15 m × 0.32 mm ID DB-1).

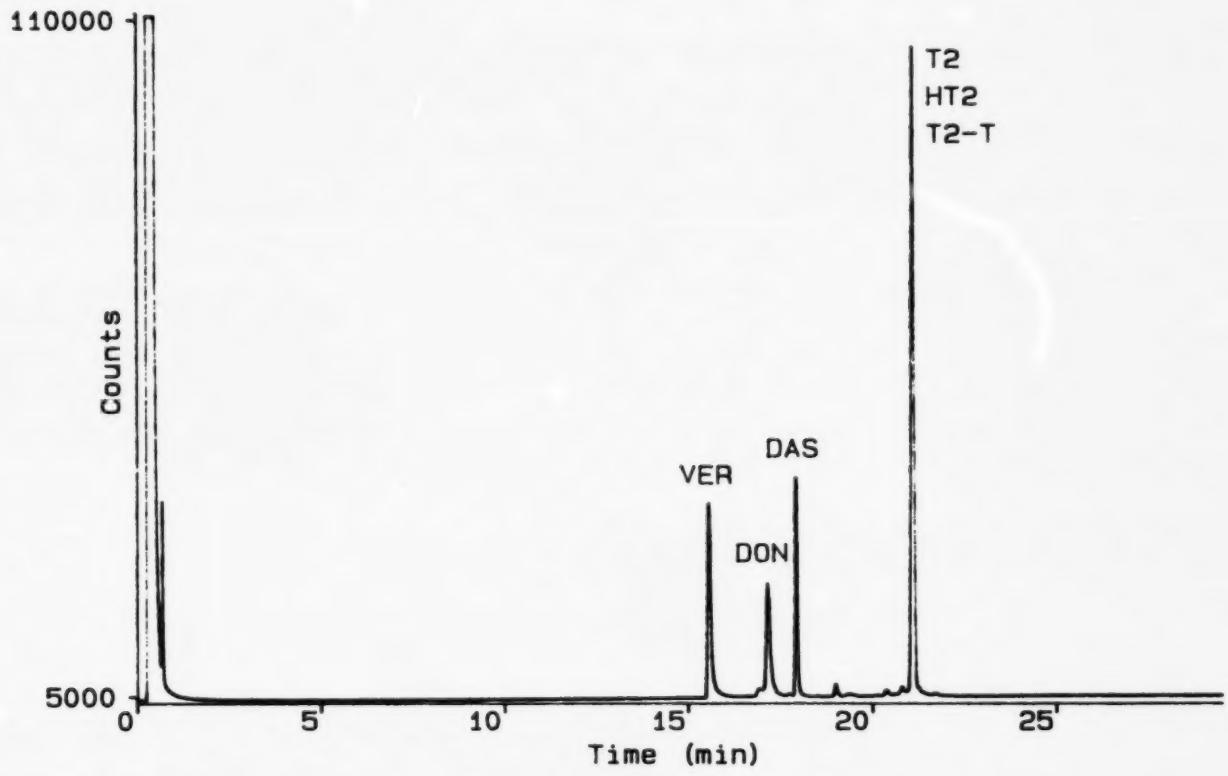


Figure 4

Capillary Column GC-FID chromatogram of 80 ng of the six mycotoxins: VER, DON, DAS, T2, HT2 and T-2 triol (T2-T) (11×10^{-11} amps full scale; 75°C (2 min) 10°/min 300°C (10 min); 15 m × 0.32 mm ID DB-1).

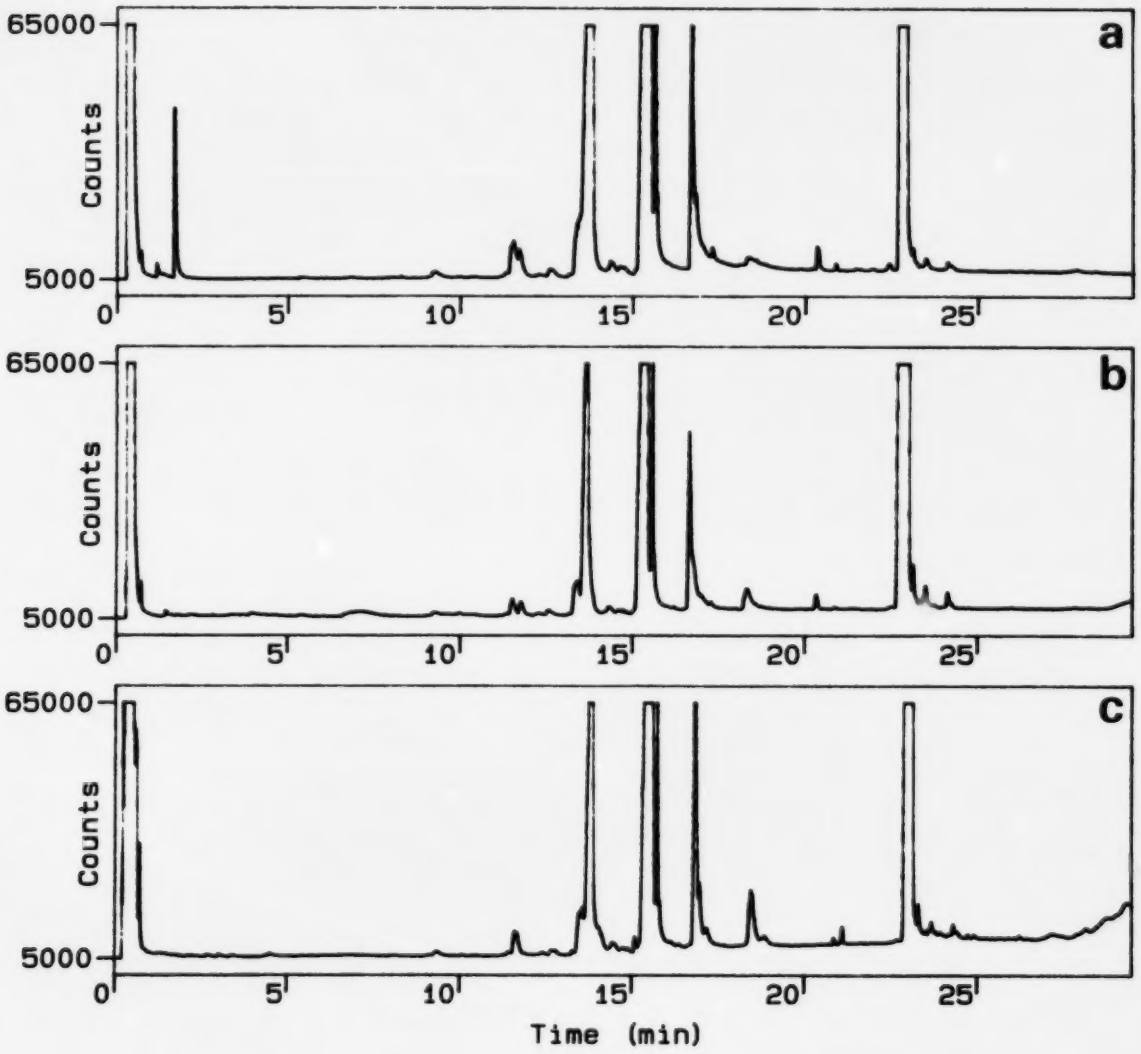


Figure 5

Capillary Column GC-FID chromatograms of blank blood taken through: a. Agriculture Canada; b. United States; and, c. United Kingdom clean-up schemes (6×10^{-11} amps full scale; 75°C (2 min) 10°/min 300°C (10 min); 15 m × 0.32 mm ID DB-1).

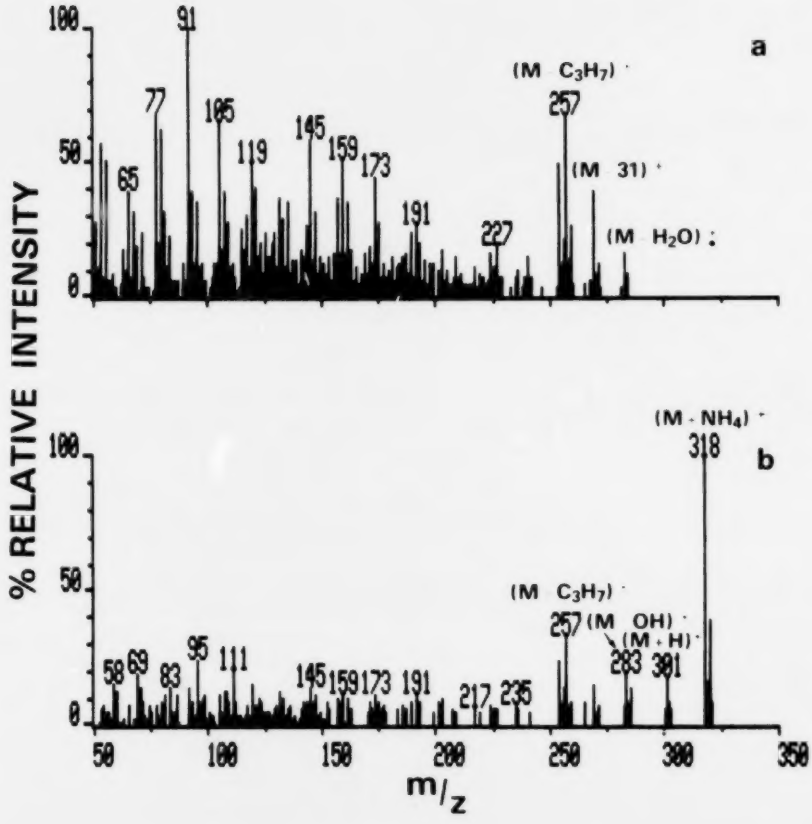


Figure 6

a) Election-impact; and, b) ammonia chemical ionization mass spectra of chlorinated-verrucarol obtained during GC-MS analysis.

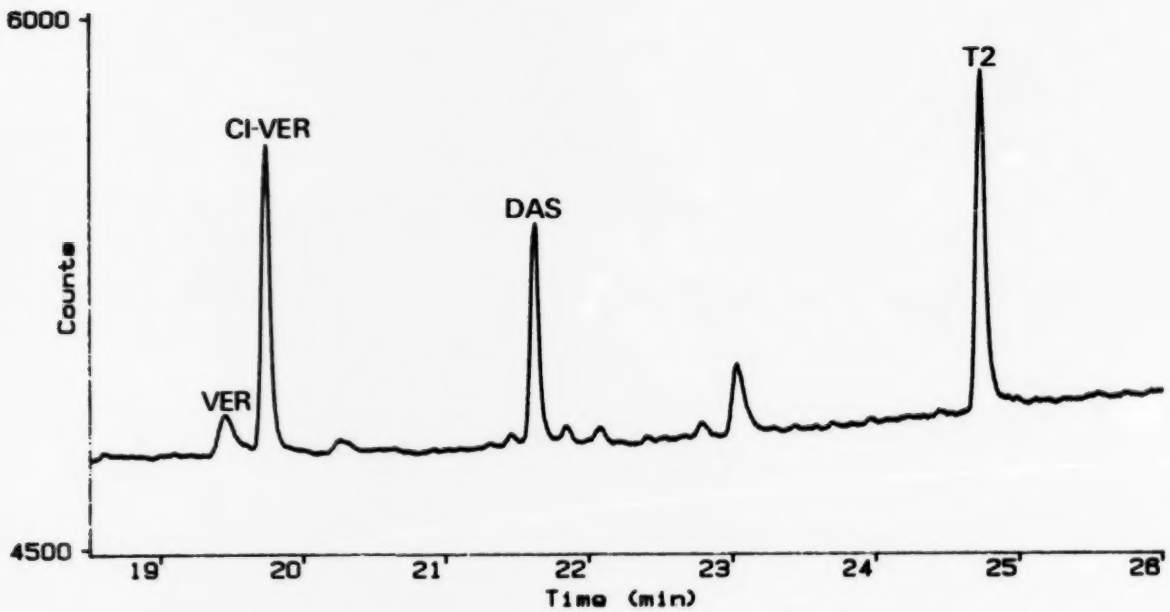


Figure 7

Capillary Column GC-FID chromatogram of a 2 μg/g spike of VER, DAS and T2 in water after the UK clean-up. Note the presence of a chlorinated-verrucarol (Cl-VER), (1.5×10^{-11} amps full scale; 50°C (2 min) 10°/min 300°C (8 min); 15 m × 0.32 mm ID DB-5).

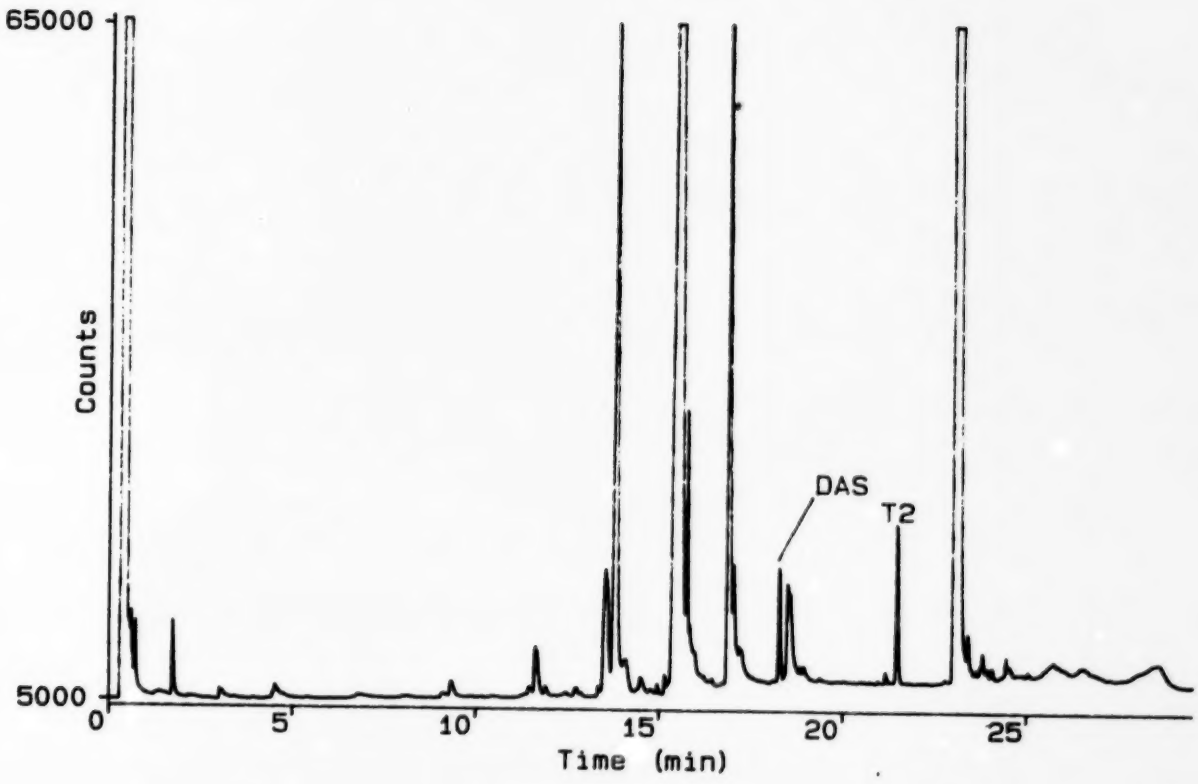


Figure 8

Capillary Column GC-FID chromatogram of a 2 μg/g spike of VER, DAS and T2 in blood after the UK clean-up. VER was not visible above the background (6×10^{-11} amps full scale; 75°C (2 min) 10°/min 300°C (10 min); 15 m × 0.32 mm ID DB-1).

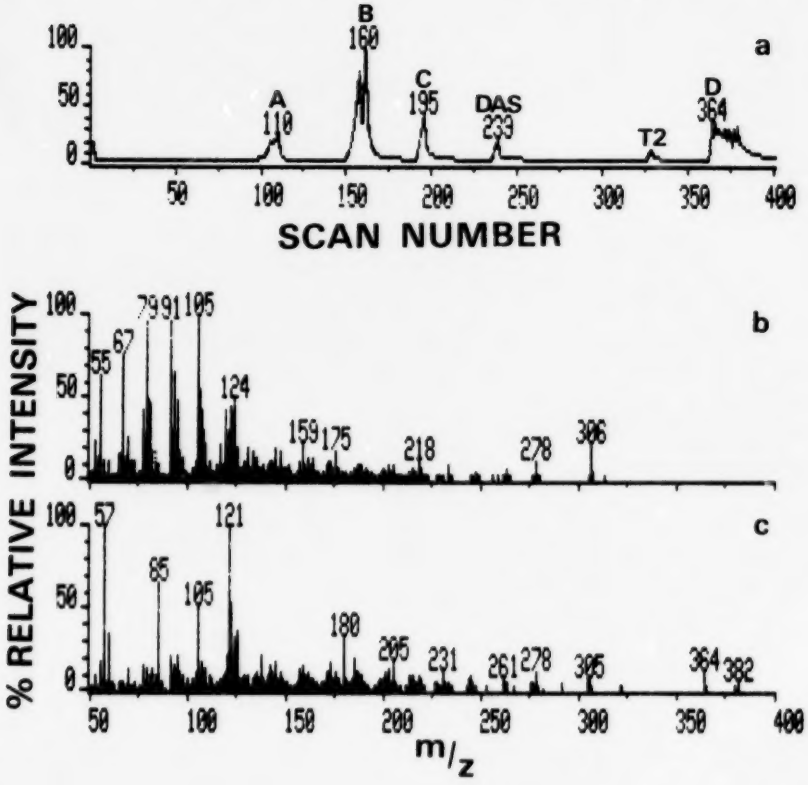


Figure 9

a) Capillary Column GC-MS (EI) total-ion-current chromatogram of 2 μg/g spike of VER, DAS and T2 in blood after the UK clean-up. The electron-impact mass spectra obtained for b) DAS and c) T2 during analysis. VER was not detected and the major blood impurities (A – D) mass spectra are illustrated in Figure 10. (50°C (2 min) 10°/min 300°C (8 min); 15 m × 0.32 mm ID DB-5;1 MS scan = 2 sec; data acquisition begins at 150°C).

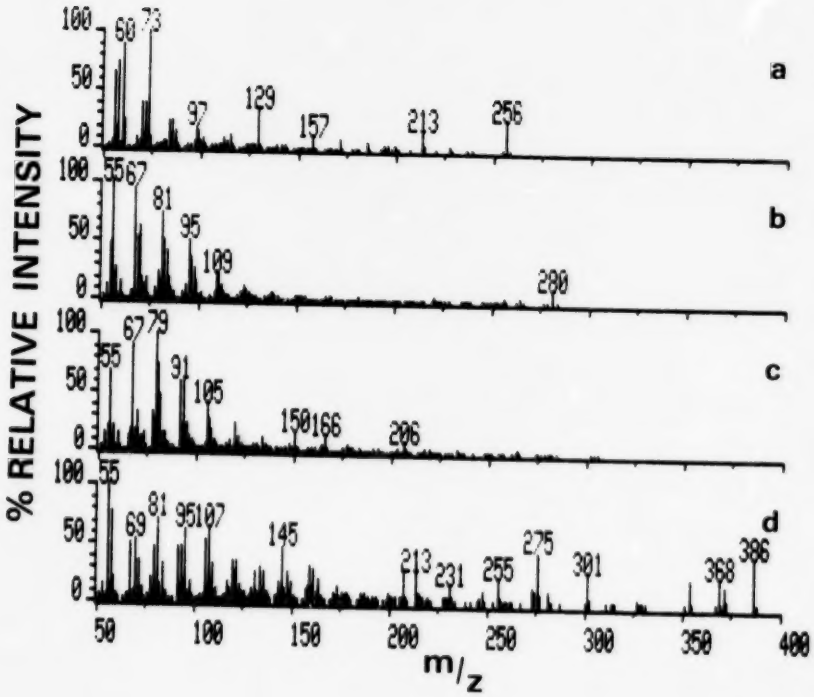


Figure 10

Electron-impact mass spectra of major blood impurities a) A, b) B, c) C, d) D identified in Figure 9.

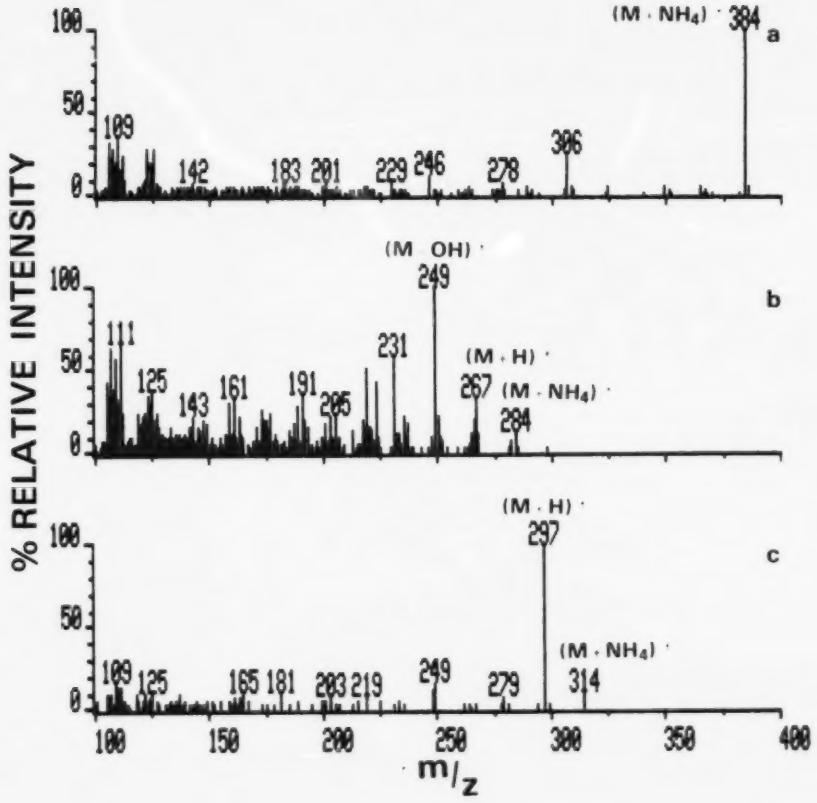


Figure 11

Ammonia chemical ionization mass spectra of:
a) DAS; b) VER; and, c) DON obtained at 160°C during GC-MS analysis.

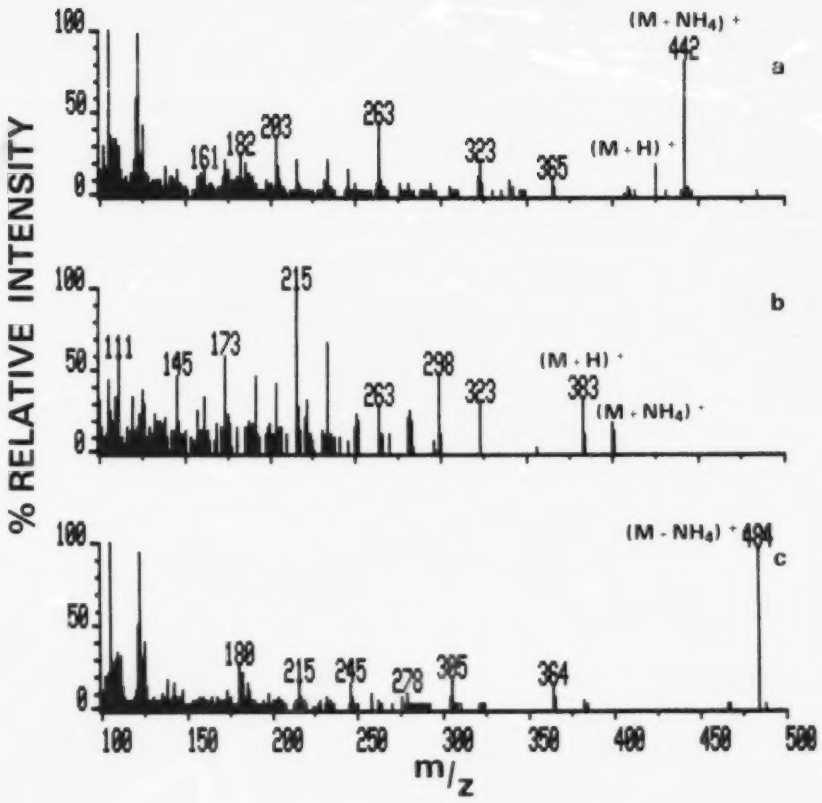


Figure 12

Ammonia chemical ionization mass spectra of:
a) HT2; b) T-2 triol; and, c) T2 obtained at 160°C during GC-MS analysis.

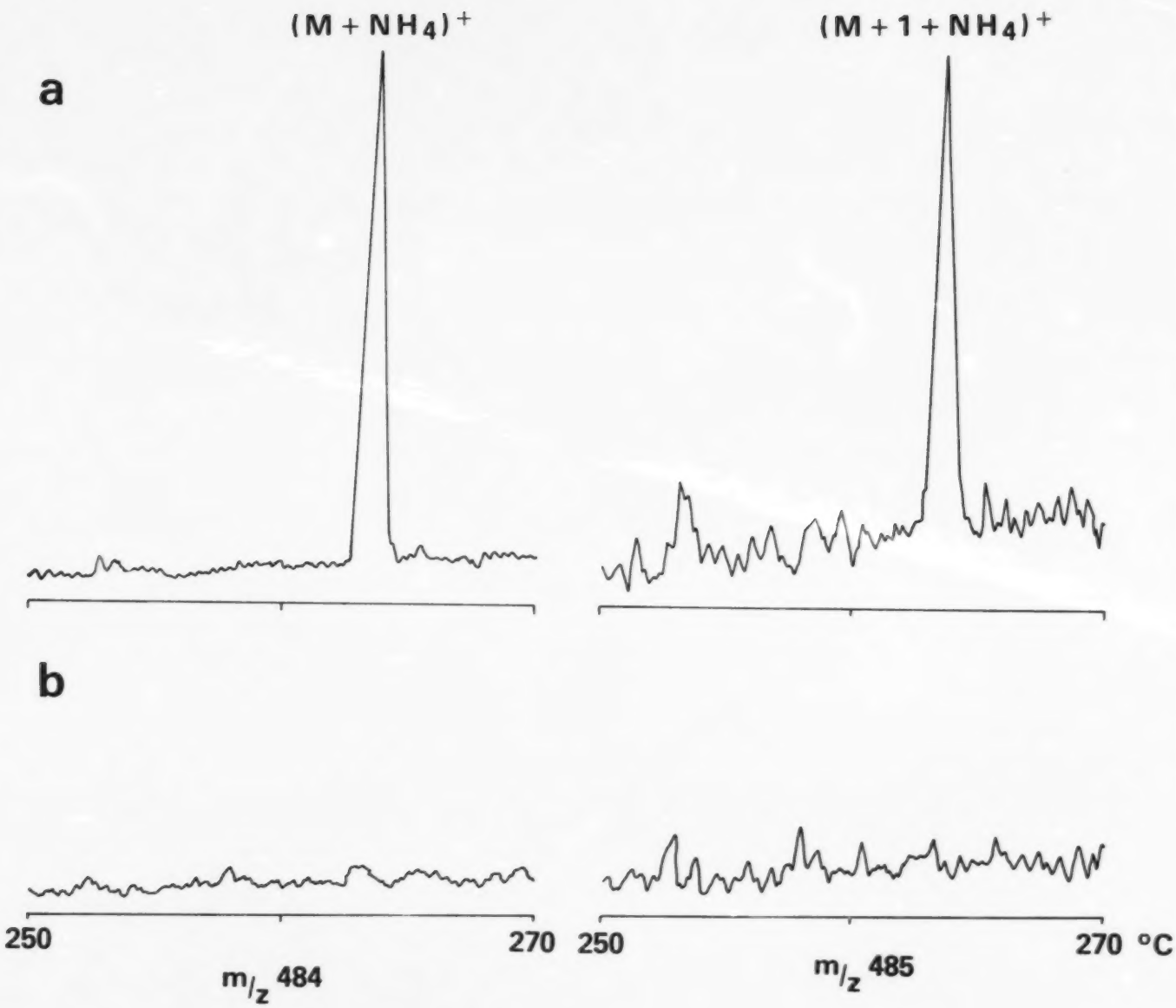


Figure 13

a) **Determination of T2 in blood at 2.2 ng/g by capillary column-ammonia chemical ionization mass spectrometry with selected-ion-monitoring of $(M+NH_4)^+$ and $(M+1+NH_4)^+$ ions.**

b) **Corresponding blood blank. (50°C (2 min) 10°/min 300°C (8 min); 15 m × 0.32 mm ID DB-5).**

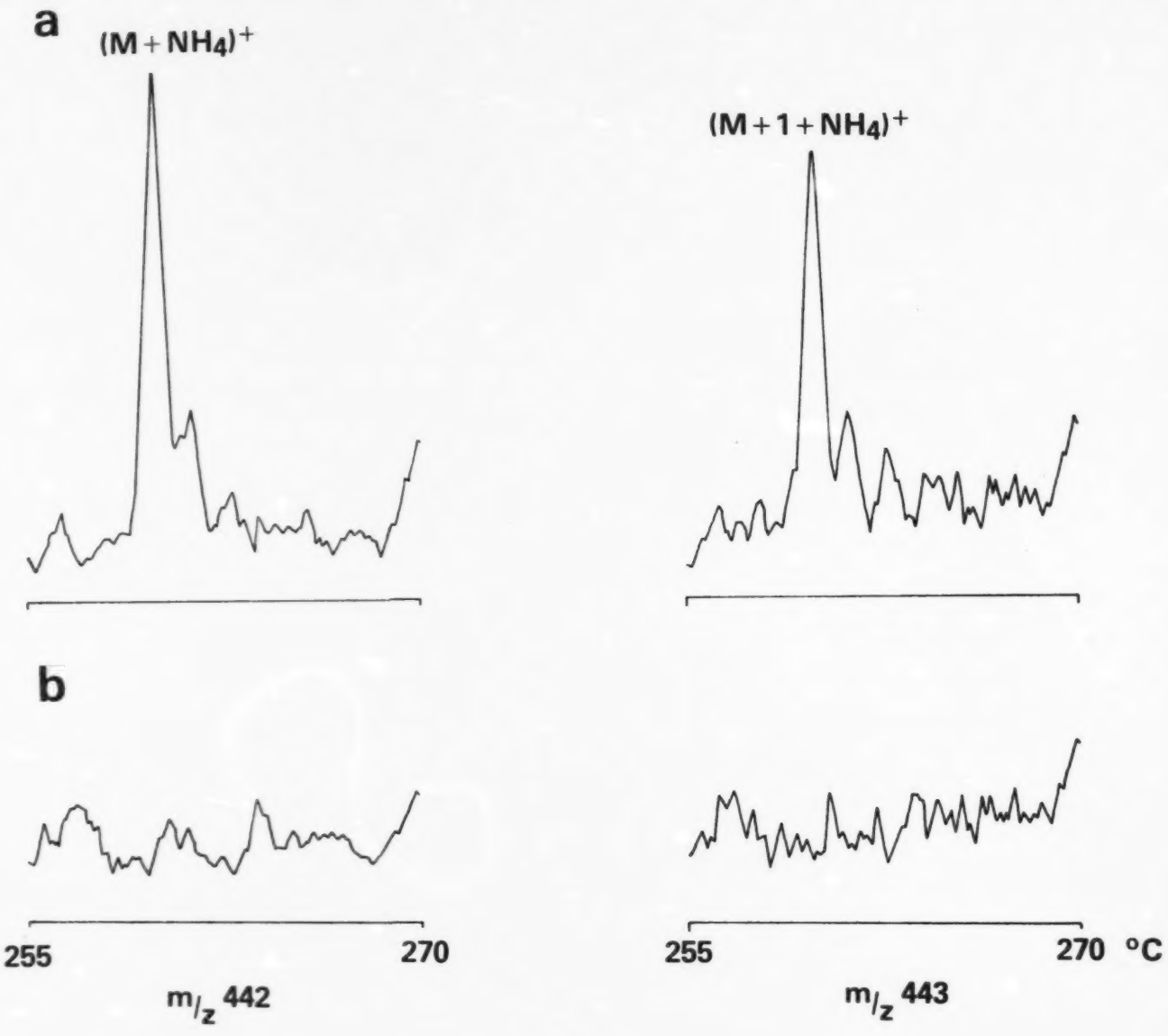


Figure 14

a) Determination of HT-2 in blood at 4.5 ng/g by capillary column-ammonia chemical ionization mass spectrometry with selected-ion-monitoring of $(M+NH_4)^+$ and $(M+1+NH_4)^+$ ions.

b) Corresponding blood blank. (50°C (2 min) 10°/min 300°C (8 min); 15 m × 0.32 mm ID DB-5).

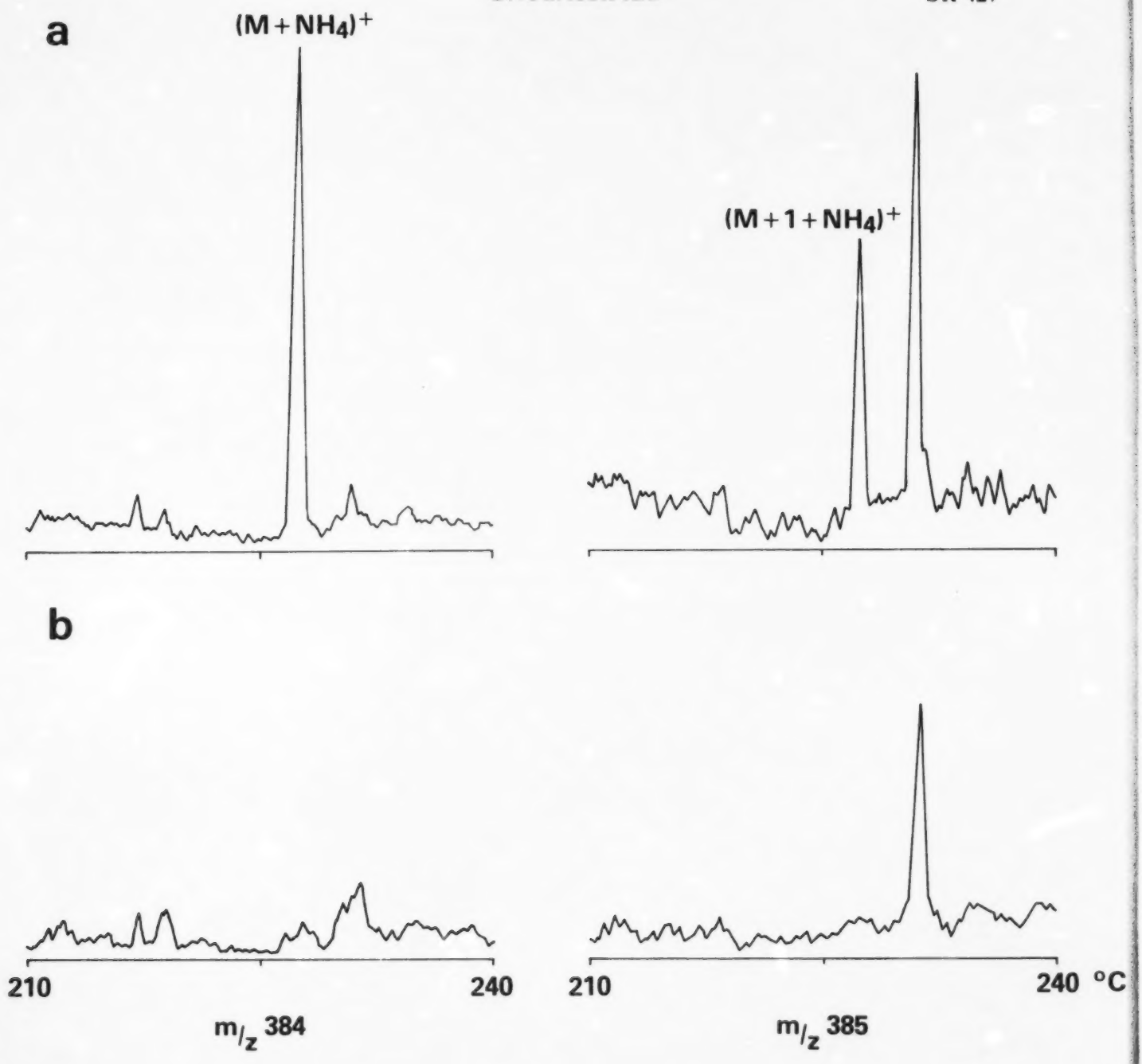


Figure 15

a) Determination of DAS in blood at 9.9 ng/g by capillary column-ammonia chemical ionization mass spectrometry with selected-ion-monitoring of $(M+NH_4)^+$ and $(M+1+NH_4)^+$ ions.

b) Corresponding blood blank. (50°C (2 min) 10°/min 300°C (8 min); 15 m × 0.32 mm ID DB-5).

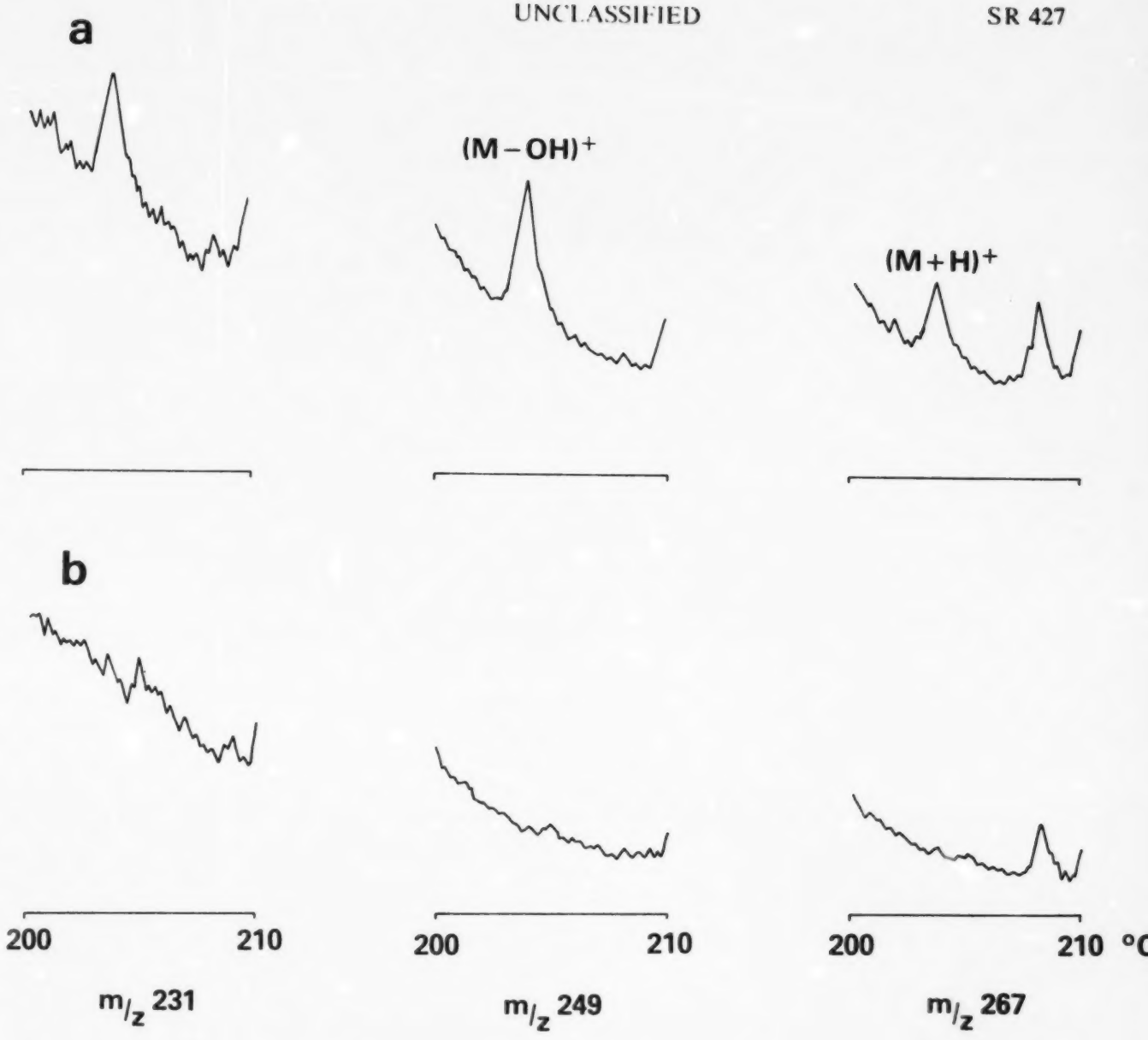
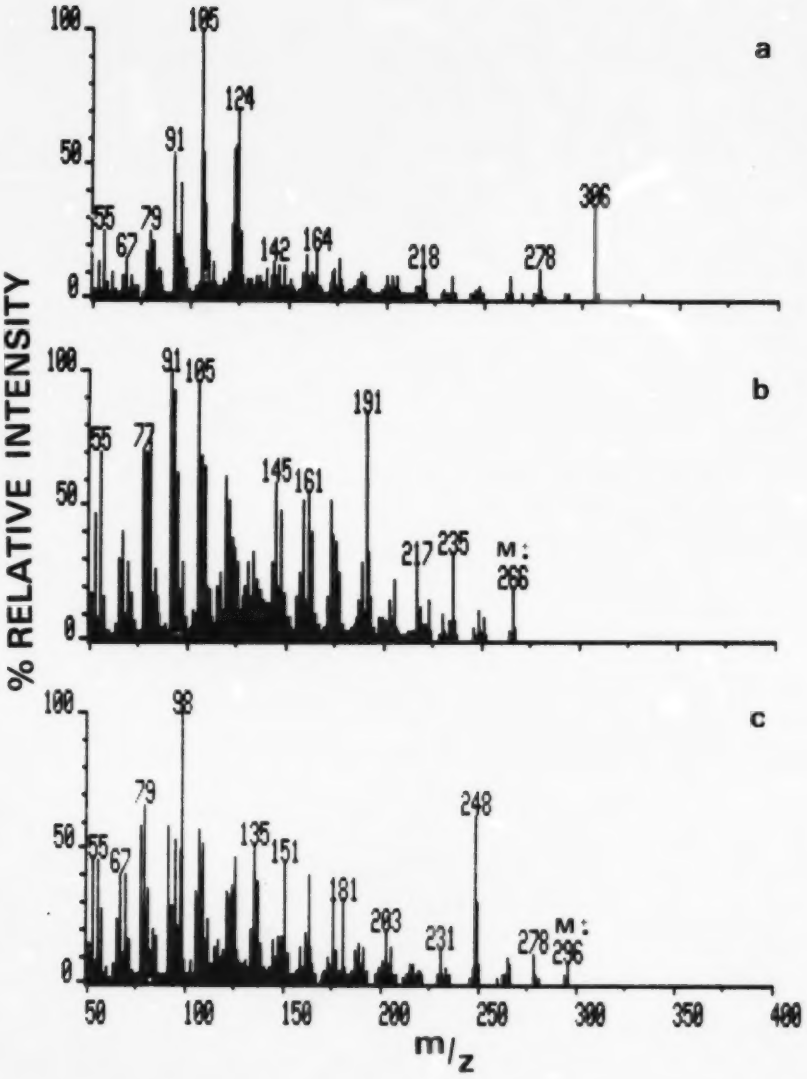


Figure 16

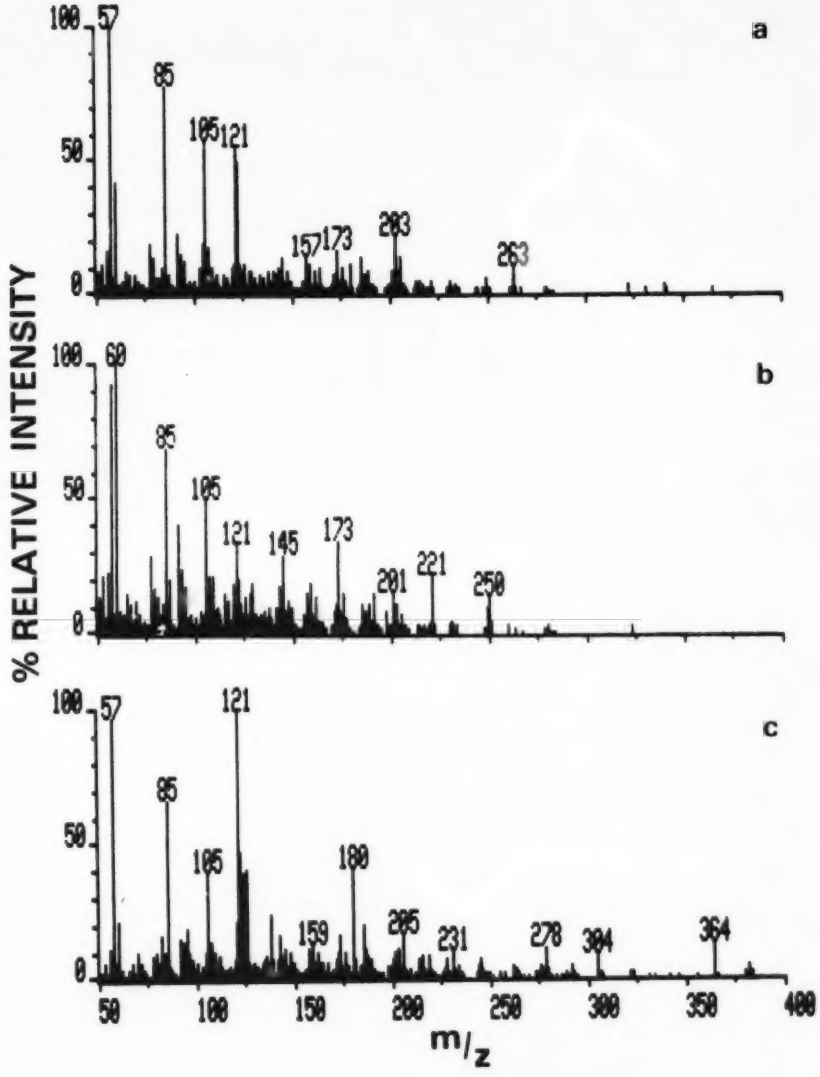
a) Determination of VER in blood at 5.1 ng/g by capillary column-ammonia chemical ionization mass spectrometry with selected-ion-monitoring of $m/_z$ 231 (a fragmentation ion), $(M-OH)^+$ and $(M+H)^+$ ions.

b) Corresponding blood blank. (50°C (2 min) 10°/min 300°C (8 min); 15 m × 0.32 mm ID DB-5).



Appendix 1

Electron-impact mass spectra of:
a) DAS; b) VER; and, c) DON obtained during GC-MS analysis.



Appendix 2

Electron-impact mass spectra of:
a) HT2; b) T-2 triol; and, c) T2 obtained during GC-MS analysis.

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Mycotoxins
Blood
T-2 toxin
HT-2 toxin
T-2 triol
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